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<p>(21) International Application Number: PCT/US93/02101</p> <p>(22) International Filing Date: 8 March 1993 (08.03.93)</p> <p>(30) Priority data: 848,087 9 March 1992 (09.03.92) US</p> <p>(71) Applicant: DIFCO LABORATORIES [US/US]; 1180 Ellsworth Road, Ann Arbor, MI 48108 (US).</p> <p>(72) Inventor: EDEN, Gideon ; 2210 Brockman Boulevard, Ann Arbor, MI 48104 (US).</p> <p>(74) Agent: KOHN, Kenneth, I.; Reising, Ethington, Barnard, Perry & Milton, P.O. Box 4390, Troy, MI 48099 (US).</p>		<p>(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published With international search report.</p>
<p>(54) Title: DIAGNOSTIC MICROBIOLOGICAL TESTING APPARATUS AND METHOD</p>		
<p>(57) Abstract</p> <p>A diagnostic microbiological testing apparatus and method includes at least one test tray (11) including a plurality of reaction chambers (12), a light source (22) disposed proximate to the test tray (11) for directing light, at an excitation wavelength of a fluorescence emitting agent contained within the reaction chambers (12), at the test tray (11), a filter for passing there-through only light generated by a fluorescence emitting reaction resulting from the interaction of the fluorescence emitting agent and a sample, and an imaging mechanism (26) for detecting only the light generated by the fluorescence emitting reaction at the emission wavelength simultaneously from the plurality of reaction chambers (12).</p> <div data-bbox="669 1150 1412 1915"> </div>		

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**DIAGNOSTIC MICROBIOLOGICAL
TESTING APPARATUS AND METHOD**

1. Technical Field

5 The present invention relates to
microbiological testing apparatus and methods
and, more specifically, to means for
susceptibility and identification testing of
samples, such as those from patients possibly
10 infected by a microbe.

BACKGROUND OF THE INVENTION

Many systems exist for conducting tests
of microbiological samples for providing patient
15 diagnosis and therapy. It is desirable to use
automated systems requiring minimal handling by a
technician. At the same time, it is also
desirable to utilize systems which provide the
most accurate results possible.

20 Such systems as described above can be
used for identification testing wherein it is
desirable to determine the identification of any
microbes present in a patient's sample.
Alternatively, or additionally, it is also
25 desirable to utilize a systems which can be used
for susceptibility testing. Susceptibility

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testing determines the susceptibility of a microbe in a sample to various therapeutics, such as antibiotics.

The U.S. Patent No. 3,297,873 to
5 Hovnanian discloses a television camera utilized with a control unit in video amplifier, a horizontal line analyzer and a TV monitor. This provides a visual read-out of micro-organisms. The TV monitor displays the specimen or a part of
10 the specimen utilizing a lens and filter combination. Furthermore, a photometer determines the UV absorption or transmission characteristics of micro-organisms, cells or other micro-specimens. The display includes a
15 darkened area, as well as a display of area. The darkened area represents the micro-sampled portion of the specimen.

The U.S. Patent No. 4,061,469 to DuBose discloses a blood analyzer which utilizes two
20 photodetectors, one as the measuring detector of the sample and the other as a reference detector. The second photodetector senses energy supplied from the source through an individual sample.

The U.S. Patent No. 4,166,095 to Kling
25 discloses an automatic chemical testing apparatus

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with visual monitoring and inputting of test results.

The U.S. Patent No. 4,175,860 to Bacus discloses a method and apparatus for classifying
5 cells, such as red blood cells. The apparatus generates an image that is split into a high resolution and a low resolution image wherein the circuitry performs measurement and analysis relating to the size, density and color of the
10 cytoplasm and the nucleus. The analysis obtained from each of the two images are applied to classification logic circuitry for the purpose of determining malignant cells. The images are obtained from a vidicom camera which are sent to
15 an analog digital converter and to a video monitor. A single slide is used.

The U.S. Patent No. 4,431,307 to Suovaniemi discloses a particular type of cuvette whose slide walls are provided with a layer of
20 material that prevent measurement of radiation or light directed at the walls for passing through the side walls. The patent discloses that a photo-measurement will be taken of each individual cuvette and the material therein.

25 The U.S. Patent No. 4,400,353 to Meseral, et al. discloses an electro-optical

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system for use in evaluating immunological reactions. Fluid biological test specimens and a reagent are introduced into a reaction zone in an image cell. The reaction cells are formed of two planar surfaces made of glass or plastic material which are provided with a generally circular groove to define the reaction cell. A fill port is pierced in a circular groove for introducing the reagent and the biological fluid. Each image cell is lifted out of its respective compartment and brought into the optical pass sequentially. After transilluminating the reaction zone and imaging light being transmitted therethrough on an image sensor, the dark areas formed on the surface of the image sensor are measured by electronics. The image sensor is a charge coupled device (CCD). When several indicator particles agglutinate, the resulting image will shadow several pixels which appear darker than a single particle. The CCD is scanned electronically row by row to obtain each pixel of information. The image areas are quantified electronically and the total area is obtained which is a function of the concentration of the antibody in the wells. The total dark images of the control specimen is related to the respective

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concentrations. The imaged data is fed to a threshold comparator and particle counter which screens the non-agglutinated particles on the basis of both intensity and particle size.

5 The U.S. Patent No. 4,453,266 to Bacus discloses a method and apparatus for measuring cell volume of a red blood cell on a slide. The apparatus includes means for generating signals representative of the area of the cells, and
10 means for measuring the optical density of the individual cells and for generating signals representative of the hemoglobin or massive cells. More than one red blood cell is determined. The image is obtained by a
15 television camera which sends this image to electronics for the analysis. Each of the several cells displayed in the image are independently analyzed.

 The U.S. Patent No. 4,580,895 to Patel
20 discloses a scanning photometer for reading agglutination tests and other procedures by scanning the contents of a micro-test well or other sample holding vessel to determine certain characteristics of the content. The patent
25 discloses scanning an entire tray having a plurality of wells and obtaining a video image

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across each well. The tray or plate which is utilized has an array of uniformly diametered, upwardly opening sample-holding wells. An XY mover is connected to the holder to move the

5 sensor assembly in a horizontal X-Y coordinate plane to successively bring wells in a preselected order to axial alignment with photodetector. The sensor assembly comprises a

10 photo beam interrupt comb which has a set of parallel and uniformly spaced apart photo beam interrupting teeth arranged in a straight row extending parallel to the motion path of the carriage in X coordinate axis. The assembly also

15 comprises a photo beam interrupt comb which has eight parallel teeth which extend in the Y coordinate axis. The combs cause the production of interrupt signals to the microprocessor to aid in the movement of the tray and designation of each well. The scanning operation is repeated

20 column for column of each of the columns in plate. The photodetector's analog output signal is a measurement of the intensity of the photometers light beam and represents a

25 continuous, traveling measurement of the optical density of the substance in diametrically across each entire well and the optical density of the

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well bottom. Twenty-four signal samples of the photodetector analog output are digitized periodically such that samples are uniformly spaced apart diametrically across each well. The digital optical density readings of each of the wells are processed by the microcomputer. The photodetector obtains a sample across a diameter of the wells, rather than the entire circle of the well. The microcomputer utilizes a threshold value in a determination of lights and darks of the sample.

The U.S. Patent No. 4,784,947 to Noeller discloses still photographing a plurality of samples at a single time.

The Japanese Patent Nos. 59-087777 and 59-78681 disclose imaging by a video camera of samples to determine optical densities therein. Single samples are imaged.

The U.S. Patent Nos. 4,720,463 and 4,856,073, both to Farber et al. relate to an apparatus and process of automatically obtaining test results from microbiological test rays. In general, microbiological sample and agent to be tested are placed in test rays having a plurality of wells or cupolas. The trays are moved to an incubator for a predetermined time. Thereafter,

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the trays are moved to an inspection station. A light source is disposed above the tray and a pair of video cameras are disposed below the tray in the inspection station. The video cameras
5 take images of the tray, well by well, and a processor processes the images to analyze the test results. The processor records the background light level of each point or pixel only within the area of interest for each
10 particular well of the tray. For each well, the image processor determines the number of pixels in the area of interest which have an associated voltage exceeding a predetermined threshold for that area of interest. If the number of pixels
15 exceeds a predetermined number, a positive result is assigned to that well. The image processor analyzes the binary partial results from the wells to determine possible identity of the micro-organisms.

20 The present invention provides a drastic simplification of the prior art apparatus which more accurately identifies and provides a susceptibility testing of a sample. The present invention utilizes a mechanically simple system
25 which utilizes a fluorescent reaction for our identification and susceptibility testing. The

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fluorescent determinations are faster and much more accurate than prior art determinations due to the high signal to noise ratio of fluorometric systems. Further, an entire tray including a plurality of wells can be imaged simultaneously, not requiring a well by well video inspection. This will increase the speed of inspection, which will provide adequate time for "real time" detection, identification and susceptibility analysis.

SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a diagnostic microbiological testing apparatus for detecting the presence of a fluorescence emitting reaction (FER) resulting from the interaction of fluorescence emitting agents (FEA) and a sample for detection, susceptibility, and identification testing, the apparatus including a test tray including a plurality of reaction chambers containing the FEA, which upon reaction with a predetermined microbe in the sample will emit light at a predetermined emission wavelength upon being illuminated by light at a predetermined excitation wavelength. A light source is

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disposed proximate to the test tray for directing light, at the excitation wavelength, at the reaction chamber. Filter means passes therethrough only light generated by the FER at
5 the emission wavelength. Video means detects only the light generated by the FER at the emission wavelength simultaneously from the plurality of reaction chambers, the filter means being disposed between the test tray and the
10 video means.

The present invention further provides a method of detecting the presence of the FER resulting from the interaction of the FEA and a sample for detection, susceptibility, and
15 identification testing by containing the FEA in the plurality of reaction chambers, directing light at the excitation wavelength at the chambers, passing through a filter only light generated by the FER at the emission wavelength,
20 and video imaging only the passed through light generated by the FER at the emission wavelength simultaneously from the plurality of reaction chambers.

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FIGURES AND THE DRAWINGS

Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

FIG. 1 is an elevational view of an apparatus made in accordance with the present invention;

FIG. 2 is a top plan view taken substantially along lines 2--2 of Figure 1;

FIG. 3 is a plan view of a test tray for use with the present invention; and

FIG. 4 is an example of susceptibility testing used in accordance with the present invention.

DETAILED DESCRIPTION OF THE INVENTION

A diagnostic microbiological testing apparatus instructed in accordance with the present invention is generally shown at 10 in the Figures. The apparatus is specifically useful for detecting the presence of a fluorescence emitting reaction (FER) resulting from the interaction of fluorescence emitting agents (FEA)

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and a sample for detection, susceptibility and identification testing.

Many FEA have been characterized for use in detection, susceptibility and

5 identification testing. The co-pending U.S. patent application Serial No. 542,115 to Thomson, filed June 22, 1990 and assigned to the assignee of the present invention discloses a two-dye technology for detecting, identifying and

10 susceptibility testing of samples. The technology includes a metabolic dye which changes in response to such environmental factors as pH or enzymatic cleavage, and an analytical dye. Examples of such FEA are medias containing

15 metabolic dyes such as resazurin, indoxyl and chloroindoxyl compounds and analytical dyes such as sulforhodamine, rhodamine B, eosin V and flourescein. Examples of such media are simple media chosen to promote the growth of

20 microorganisms tested. The media may contain carbohydrates. It may be made to grow specific microbes or a broad spectrum of microbes. Such media are well known in the art, such as Mueller-Hinton, Columbian broth, Schaedler's broth, Brain

25 heart broth, and tryptic soy broth.

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Samples to be tested in accordance with the present invention and utilizing the apparatus made in accordance with the present invention can be various solid or fluid samples taken from a
5 patient. The samples can be in the form of blood samples, plasma samples, spinal fluid samples or the like. Of course, the present invention could be used for veterinary and other purposes.

Generally, the apparatus 10 includes a
10 plurality of reaction chambers in the form of test tray 11. As shown in Figure 2, the apparatus 10 can include a plurality of test trays 11 contained within a carousel 14 which is effectively a rotary table rotated by an
15 actuating and indexing mechanism 16. The carousel 14 is rotated to be able to index one of the test trays 11 proximate to a detection area 18, as shown in Figure 2. Positioning means 20, such as a reciprocating arm mechanism, positions
20 one of the test trays 11, proximate to the detection area 18, into and out of the detection area for purposes as explained below. That is, the carousel 14 would allow in and out sliding movement of a test tray 11 as actuated by the
25 positioning mechanism 20 into and out of the detection area 18.

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A light source, schematically shown at 22 in Figure 2, is disposed proximate to the test tray 11 disposed in the detection area 18. The light source 22 is a high energy source which can excite fluorogenic agents at specific bands such as a tungsten lamp with a narrow band pass filter, gas filled electron discharge tubes or lasers. A filter 24 is shown to be disposed on the side of the test tray 11 opposite to the light source 22. The filter 24 is of the type that passes therethrough only light generated by the FER at the emission wavelength. That is, the filter 24 filters out light at all other wavelengths than the emission wavelength from passing therethrough. Thusly, the only light detectable beyond the filter 24 is light generated by the FER. All other images, such as an image of the test tray 11, are not detectable beyond the filter 24. Further, all of the reaction chambers 12 are detectable if an FER is present therein. Otherwise, even the reaction chamber 12 is not detectable as it will emit no detectable light beyond the filter 24.

The apparatus 10 includes a video mechanism 26, the filter 24 being disposed between the video mechanism 26 and test tray 11.

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Moreover, the only light detectable by the video mechanism 26 is the light which passes through to the filter 24. Thusly, the video mechanism 26 is only exposed to light generated by the FER at the emission wavelength. By using the filter 24, a video mechanism (two-dimensional optical detector) such as a diode array or CCD device can be used which receives, detects, and produces an image from a broad spectrum of wavelengths but the filter 24 will only expose the video mechanism 26 to the light emitted by the FER at the emission wavelength. Thusly, the video mechanism 26 will detect only the light emitted by the FER and will not image any other objects illuminated by the light source 22, such as the reaction chamber 12. Further, the video mechanism 26 will image all FER simultaneously when the test tray 11 is disposed at the detection area 18. Thusly, a series of reaction chambers 12 can be imaged, unlike prior art systems which must scan each reaction chamber separately.

The above components of the apparatus 10 are contained within a body portion 28, having a lid member 30. The body portion 28 and lid 30 completely isolate outside light sources from the

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detecting system comprising the light source 22,
test tray 11, filter 24 and video mechanism 26.

Since the reactions occurring in the
test trays 11 need to be controlled with regard
5 to temperature, the apparatus 10 can include a
temperature control and display schematically
shown at 32 in Figure 1. The apparatus also
includes control electronics schematically shown
at 34 for controlling the operation of the
10 carousel 14, camera 26 and positioning mechanism
16.

The present invention further provides
a method of detecting the presence of the FER
resulting from the interaction of the FEA and the
15 sample for detection, susceptibility and
identification testing. Specifically, the method
includes the steps of containing the FEA in a
plurality of reaction chambers 12, the FEA upon
reaction with a predetermined microbe in a sample
20 contained within the reaction chamber 12 emitting
light at the predetermined emission wavelength
upon being illuminated by light at the
predetermined excitation wavelength. Light is
directed at the excitation wavelength from the
25 light source 22 to the reaction chambers 12. A
filter 24 passes therethrough only light

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generated by the FER at the emission wavelength
and the passed through light generated by the FER
at the emission wavelength from the plurality of
reaction chambers 12 is simultaneously detected
5 by the video mechanism 26.

The following example demonstrates the
ability of the present invention to perform
susceptibility testing. The inoculum which is
comprised of: growth media (Mueller Hinton base
10 at g/L), fluorogenic substance (sulforhodamine
101 at 10 μ M), reaction dye (resazurin at 20 μ M),
antimicrobial agent (Aztreonam at various
concentrations) and Providencia alcalifaciens at
5x10⁵ cfu/ml, is introduced to multiple reaction
15 chambers with the capacity of 50 μ l each. The
test plate is incubated by setting the carousel
temperature to 35°C, and amplitude readings are
taken every 6 minutes. Figure 4 illustrates the
time curves of the various samples in which
20 curves #2, 3 and 4 indicate growth of
microorganisms due to the low concentration of
antibiotics (neg. control, 4, 8 μ g/ml.) in the
corresponding chambers. Curve #1 shows the
growth inhibition of the microorganisms in the
25 presence of the higher antibiotic concentration

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(the Minimal Inhibition Concentration - MIC) of
16 units.

The invention has been described in an
illustrative manner, and it is to be understood
5 that the terminology which has been used is
intended to be in the nature of words of
description rather than of limitation.

Obviously many modifications and
variations of the present invention are possible
10 in light of the above teachings. It is,
therefore, to be understood that within the scope
of the appended claims wherein reference numerals
are merely for convenience and are not to be in
any way limiting, the invention may be practiced
15 otherwise than as specifically described.

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CLAIMSWhat is Claimed is:

1. A diagnostic microbiological
5 testing apparatus (10) for detecting the presence
of fluorescence emitting reaction (FER) resulting
from the interaction of fluorescence emitting
agents (FEA) and a sample for susceptibility and
identification testing, said apparatus (10)
10 comprising:
a plurality of reaction chambers (12)
containing the FEA, which upon reaction with a
predetermined microbe in the sample will emit
light at a predetermined emission wavelength upon
15 being illuminated by light at a predetermined
excitation wavelength;
a light source (22) disposed proximate
to said plurality of reaction chambers (12) for
directing light, at said excitation wavelength,
20 at said plurality of reaction chambers (12);
filter means (24) for passing
therethrough only light generated by the FER at
the emission wavelength; and

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video means for detecting only the light generated by the FER at the emission wavelength simultaneously from said plurality of reaction chambers (12) and forming an image thereof, said filter means being disposed between said test tray (11) and said video mechanism (26).

2. An apparatus of claim 1 including a test tray (11) including said plurality of reaction chambers (12).

3. An apparatus as set forth in claim 2 further including a detection area (18), carousel means (14) for containing a plurality of said test trays (11), indexing means for sequentially moving said carousel (14) to selectively position each of said test trays (11) proximate to said detection area (18), and positioning means (20) for positioning one of said test trays (11) proximate to said detection area (18) into and out of said detection area (18).

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4. An apparatus as set forth in claim
1 further including reaction media for reacting
with a predetermined microbe to emit the light at
said emission wavelength, said light detection
5 means detecting the presence of the microbe by
detecting said light at said emission wavelength.

5. An apparatus as set forth in claim
1 wherein said reaction chambers (12) further
include an antibiotic for susceptibility testing.

10 6. A method of detecting the presence
of a fluorescence emitting reaction (FER)
resulting from the interaction of fluorescence
emitting agents (FEA) and a sample for
susceptibility and identification testing, said
15 method including the steps of:

containing the FEA in a plurality of
reaction chambers (12), the FEA upon reaction
with a predetermined microbe in the sample
emitting light at a predetermined emission
20 wavelength upon being illuminated by light at a
predetermined excitation wavelength;

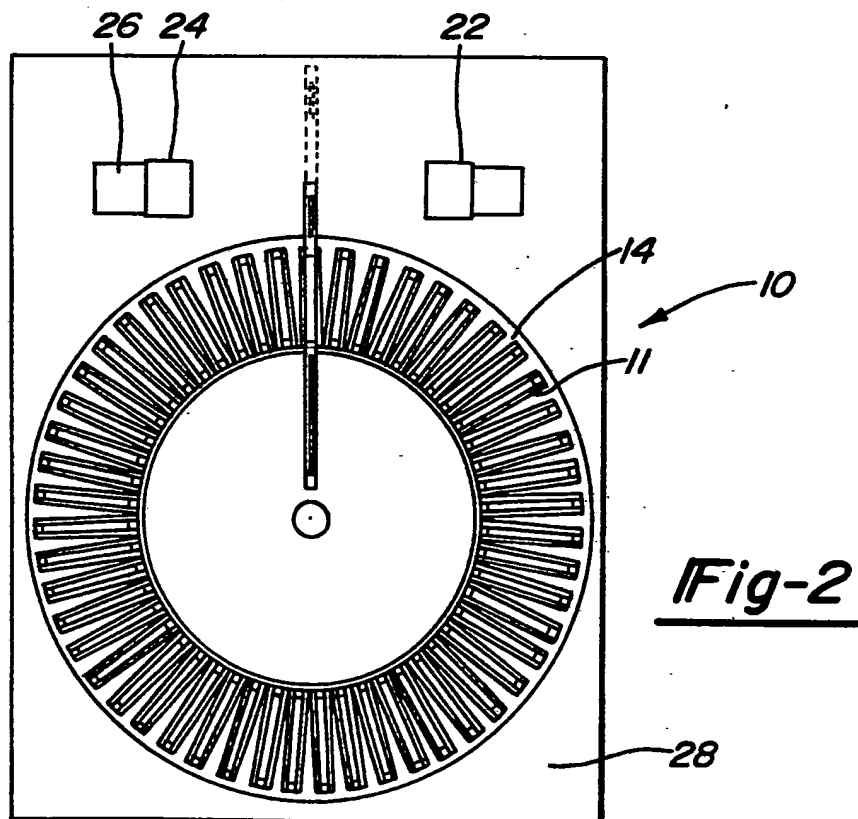
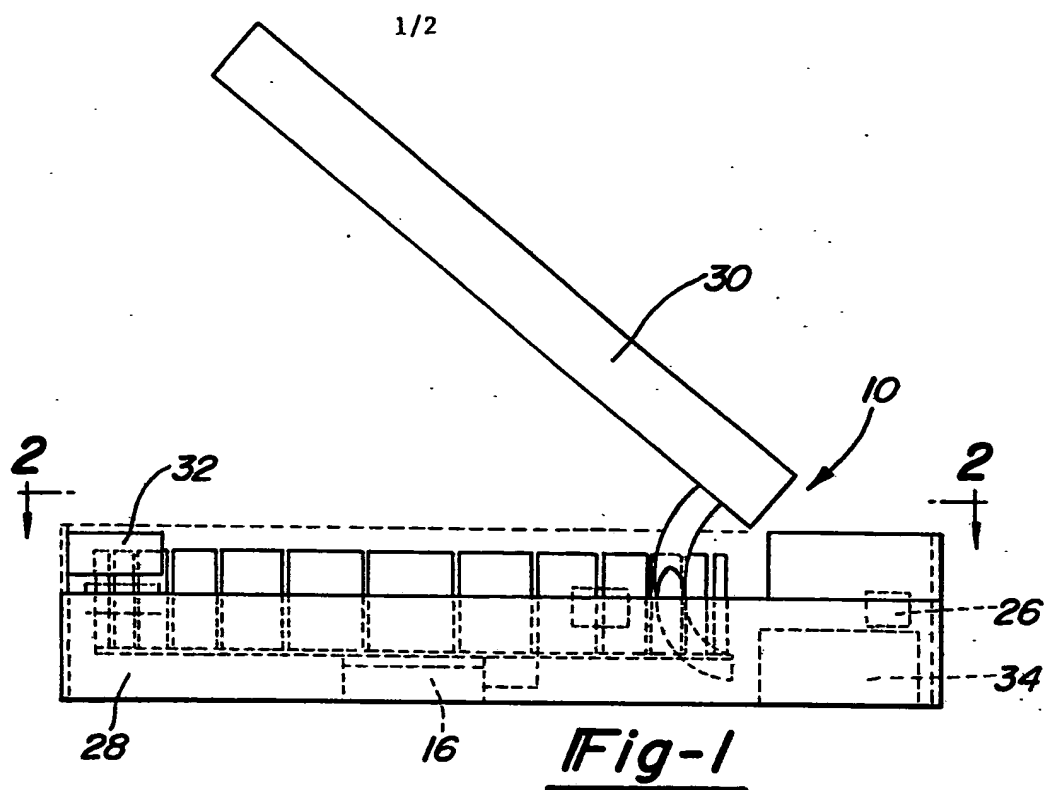
directing light at the excitation
wavelength at the plurality of reaction chambers
(12);

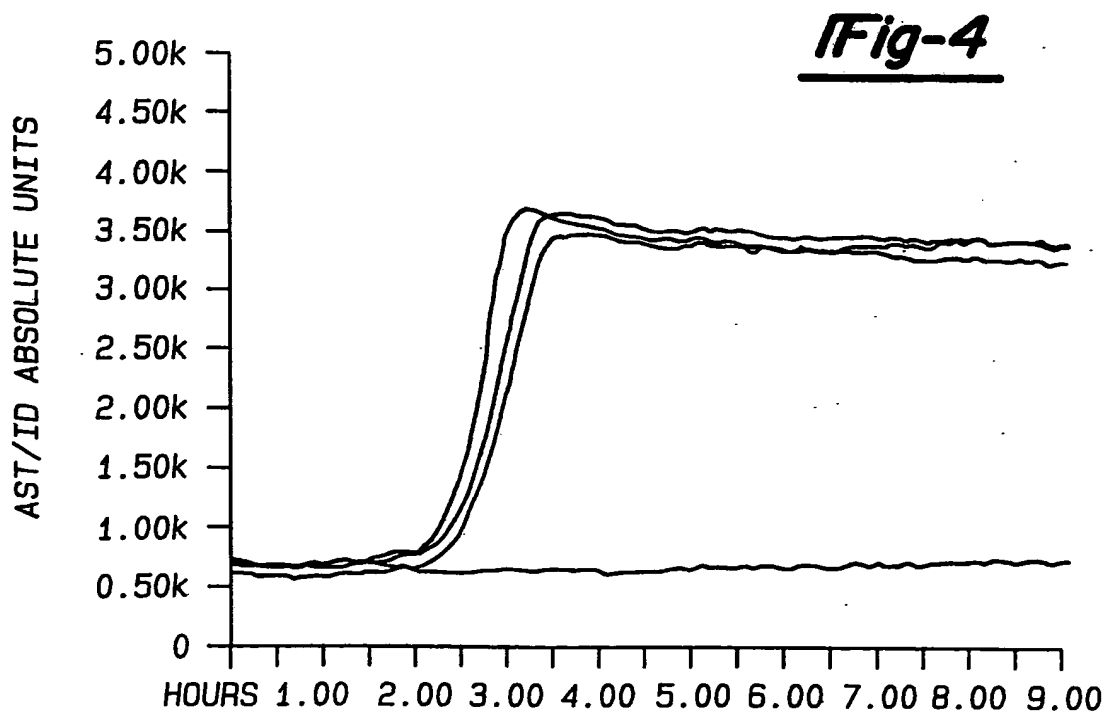
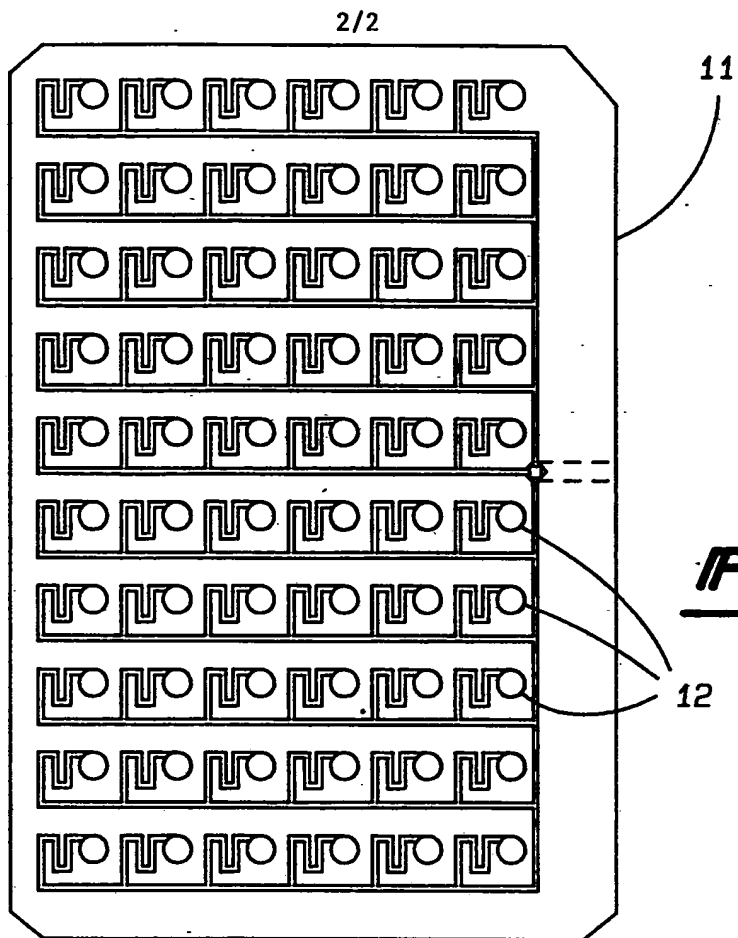
25

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passing through a filter only light generated by the FER at the emission wavelength simultaneously from the plurality of reaction chambers (12); and

- 5 video imaging only the passed through light generated by the FER at the emission wavelength simultaneously from the plurality of reaction chambers (12).





INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US93/02101

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 250/328, 361C, 461.2; 356/417; 422/52, 58, 63, 64, 67; 435/29, 31, 32, 33, 39, 289, 291, 808, 809; 436/45, 46, 172

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS: Fluorescent Emitting Reaction/Agents

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,940,332 (Miwa et al.) 10 July 1990, Figs. 2-4, Col. 2, 57-col. 3, 4 and Col. 3, 62-col. 4, 12.	1-6
Y	US, A, 4,856,073 (Farber et al.) 08 August 1989, Figs. 1, 5 and 12, Col. 4, 18-46, Col. 5, 11-37 and Col. 14, 49-64.	1-6
Y	US, A, 4,695,727 (Brierley et al.) 22 September 1987, Fig. 4, Col. 4, 59-col. 5, 9.	3
Y	US, A, 5,064,756 (Carr et al.) 12 November 1991, Col. 2, 45-col. 3, 5 and Col. 3, 24-45.	5

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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INTERNATIONAL SEARCH REPORTInternational Application No.
PCT/US93/02101**C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,956,148 (Grandone) 11 September 1990.	3

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US93/02101

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

C12Q 1/06, 1/02, 1/18; C12M 1/34, 1/36; G01N 21/00, 21/64, 21/76, 31/00, 31/22, 33/00, 35/00

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/39, 29, 32, 291, 808; 250/461.2; 422/52, 58, 63, 64; 436/46, 172